Flame Photometric Determination, Extraction, and Cleanup Procedures for GS-13005 [*O,O*-Dimethyl-*S*-2-methyloxy-1,3,4-thiodiazol-5-(4*H*)-onyl-(4)-methyl-dithiophosphate (4)] and Metabolite Residues in Egg Yolk and Poultry Feed

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A flame photometric detection method with the extraction and cleanup procedures is described for the residue analysis of Supracide GS-13005 (organophosphorus insecticide) including the oxygen analog and three metabolites, in samples containing approximately 5 to 35% fat. The method has proved satisfactory for the determination of Supracide in egg yolk and poultry feed. Fifteen samples a day have been determined over an extended period (6 weeks or more) without contamination of the column or detector. Levels of the insecticide as low as 0.002 p.p.m. were detected.

A considerable amount of data has appeared concerning the detection of organophosphorus pesticide residues by use of the flame photometric detector for samples relatively low in fat content as shown by Beroza and Bowman (1966), Bowman and Beroza (1966, 1967), and Getz (1967). Few publications indicate the usefulness of the detector for the detection of sulfur-containing compounds as described by Brody and Chaney (1966) or samples containing large amounts of fatty material.

Eberle *et al.* (1967) reported the detection of GS-13005 using GLC (electron-capture), thin-layer chromatographic, and colorimetric procedures for fruit and cotton samples at the 0.01-p.p.m. level.

Although the methods described above were satisfactory for samples low in fat content, the author was unable to extend them to samples containing high fat concentrations, low residue levels, or sulfur-containing residues and their metabolites. Metabolites that one would expect to find (sulfides, sulfones, and sulfoxides) indicate that the phosphorus end of the GS-13005 molecule is split off, resulting in the ultimate formation of a sulfur compound requiring the use of the sulfur filter rather than the phosphorus filter.

MATERIALS AND METHODS

Reagents and Solvents. Anhydrous granular sodium sulfate (Mallinckrodt), attapulgus clay (Minerals and Chemicals Corp. of America), activated carbon (Nuchar C 190 N, Fisher Scientific Corp.) and aluminum oxide (Merck No. 71707) were used from the suppliers listed, as other sources of supply contained interfering substances. The column packing was mixed and packed in the laboratory and contained 3% SE-30 and 3.3% Epon on Gas-Chrom Q.

Organic solvents, acetonitrile, hexane, petroleum ether, and dichloromethane were redistilled in glass in the laboratory to obtain clean chemicals.

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Technical standard GS-13005 98.2%, oxygen analog, and primary metabolites GS-28368 (sulfide), GS-28369 (sulfone), and GS-28370 (sulfoxide), shown in Figure 1, were obtained from Geigy Chemicals.

Apparatus. A MicroTek Gas Chromatograph (Tracor, Inc.) equipped with the Melpar flame photometric detector having interference filters for phosphorus (526 mμ) and sulfur (394 mμ) of Brody and Chaney (1966) for the detection of GS-13005 was used.

Extraction of Egg Yolk. Four grams of egg yolk were weighed into a 200-ml. stainless steel Omni-Mixer blender cup, spiked (for standards) and blended with 20 grams of

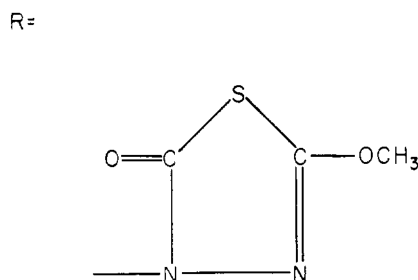

Figure 1. Structural formulas for Supracide, oxygen analog, and metabolites

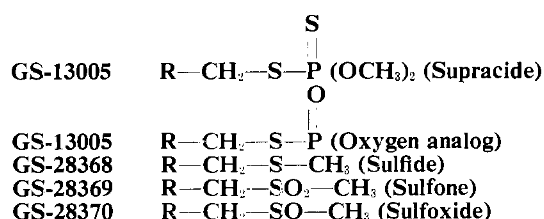

Table I. Recoveries of GS-13005 in Feed and Egg Yolk

(Retention time, 8 min.)

Feed[a]	Lab. No.	Sample Size p.p.m.	Per Cent Recoveries from 3 Replications I	II	III	Average Recovery %	p.p.m.
Part I	1	Control	0	0	0	0	0
	2	2.5	93.2	79.5	88.9	87.2	4.36
	3	5.0	91.7	75.9	89.2	85.6	6.42
	4	10.0	87.0	86.8	86.8	86.9	10.86
	5	15.0	93.5	93.5	90.1	92.4	16.17
	6	20.0	88.9	97.6	88.9	91.8	18.36
						Mean 88.8	
Part II[b]	1	Control	0	0	0	0	0
	2	2.5	79.5	84.4	93.2	85.7	4.29
	3	5.0	81.7	87.0	91.7	86.8	6.51
	4	10.0	94.2	87.0	88.3	89.8	11.23
	5	15.0	93.5	91.1	89.2	91.3	15.98
	6	20.0	89.5	88.9	92.6	90.3	18.01
						Mean 88.8	
Egg Yolk[a]							
Part I	1	Control	0	0	0	0	0
	2	2.5	88.2	88.2	90.2	88.9	2.23
	3	5.0	97.2	88.4	93.1	92.9	4.65
	4	7.5	91.7	95.0	93.0	93.2	7.00
	5	10.0	95.2	95.2	95.2	95.2	9.52
	6	15.0	95.2	89.1	89.1	91.1	13.67
	7	20.0	91.3	91.3	97.6	93.4	18.68
						Mean 92.4	
Part II[b]	1	Control	0	0	0	0	0
	2	2.5	88.2	89.7	90.1	89.3	2.23
	3	5.0	77.2	87.2	89.1	84.5	4.23
	4	7.5	95.2	96.0	96.8	96.0	7.20
	5	10.0	82.6	88.1	84.1	84.9	8.41
	6	15.0	91.3	91.8	92.6	91.9	13.79
	7	20.0	88.9	93.5	94.4	92.3	18.46
						Mean 90.0	

[a] Phosphorus filter, 526 mμ.
[b] Sulfur filter, 394 mμ.

anhydrous sodium sulfate for 1 minute. One hundred milliliters of petroleum ether were added to the chamber and blended for 1 minute. The sample was allowed to stand for 10 minutes, the liquid decanted into a beaker and filtered through a 25 mm. × 28 mm. column of anhydrous sodium sulfate. The mixer chamber and beaker were rinsed with 150 ml. of petroleum ether and poured through the above column. The rinsings were combined with the sample and stored, under refrigeration, for the cleanup procedure.

Extraction of Poultry Feed. One hundred grams of feed were weighed into a 200-ml. stainless steel Omni-Mixer blender cup, spiked (for standards) and 100 ml. of petroleum ether were added and mixed for 1 minute at three-fourth speed. The petroleum ether was decanted into a beaker and poured through a 25 mm. × 28 mm. column of anhydrous sodium sulfate. The blender cup, beaker, and column were rinsed with 200 ml. of petroleum ether and the sample was saved for the cleanup procedure.

Cleanup Procedure for Egg Yolk and Feed. The sample (petroleum ether layer) was evaporated on a Rotary flash evaporator to *ca.* 50 ml. It was then transferred to a 250-ml. separatory funnel using petroleum ether as a wash and to adjust the volume to *ca.* 100 ml.

The petroleum ether was partitioned by shaking it gently with seven 10-ml. portions of acetonitrile. The acetonitrile was saved in a 125-ml. separatory funnel and washed twice with two 50-ml. portions of petroleum ether.

The combined acetonitrile sample was evaporated to dryness in a Rotary flash evaporator and taken up in 25 ml. of 1% methanol–methylene chloride, poured onto a No. 5 column (Samuel, 1966) and eluted from the column with 175 ml. of 1% methanol–methylene chloride. The No. 5 column consisted of *ca.* a 20-mm. layer of No. 5 mixture overlaid with a 5-mm. layer of anhydrous granular sodium sulfate, in borosilicate glass 30-ml. coarse Buchner funnel. An 8:8:2:3:6 ratio by weight of a homogeneous mixture, consisting of anhydrous granular sodium sulfate, Celite 545, attapulgus clay, Nuchar 190 N activated carbon, and Merck No. 71707 aluminum oxide, was used in making the No. 5 column mixture.

To remove the metabolites from the sample, leaving only the parent compound, the sample was run through a prepared 3.5% H_2O Florisil column (the 10 cm. × 20 mm. Florisil column was composed of a 5-mm. layer of anhydrous sodium sulfate on the bottom and top of the Florisil). The column was prewashed with 100 ml. of petroleum ether and 50 ml. of 1% methanol–methylene chloride. The sample beaker and column containing the sample were washed with 150 ml. of 1% methanol–methylene chloride.

The sample was then evaporated to dryness on a Rotary flash evaporator, taken up in hexane to the desired volume and injected onto the gas chromatography column.

Gas Chromatography. The following conditions were used in the analysis with the flame photometric detector: Column,

150 cm. × 5 mm. I.D. glass; packing 3% SE-30 and 3.3% Epon on 80- to 100-mesh Gas Chrom Q (Applied Science Labs., State College, Pa.) preconditioned 48 hours at 230° C.; gases, nitrogen (carrier) at 80 ml. per minute, oxygen at 200 ml. per minute, and hydrogen at 150 ml. per minute with tank pressures at 40 pounds; temperature, column 180° C., injection port 220° C., detector 155° C.; attenuation, input 10^4, output 16.

The column was easily conditioned for GS-13005 by injection of six 10-μl. portions (100 ng. each) of the insecticide onto the column. When the column was equilibrated and the conditions were standardized, the samples were injected. Standards were checked daily before and after analysis of samples. The samples were run on the flame photometric detector using interference filters for the spectral isolation of the phosphorus emission at 526 mμ and the sulfur emission at 394 mμ.

RESULTS AND DISCUSSION

The procedures given above may be used for the extraction, cleanup, and analysis of the parent compound GS-13005, the oxygen analog GS-13007, and the metabolites (GS-28368, a sulfide; GS-28369, a sulfone; and GS-28370, a sulfoxide) in samples containing from 5 (poultry feed) to 35% (egg yolk) fat.

Straight standard curves were obtained with the pure insecticide, spiked poultry feed, and spiked egg yolk. The retention times on the gas chromatography column were GS-13005, 8 minutes; GS-13007, 8.15 minutes; GS-28368, 1.69 minutes; GS-28369, 2.50 minutes; and GS-28370, 3.20 minutes. The feed and egg yolk standards were spiked with 2.5, 5, 10, 15, and 20 μg. per ml. of the standard and analyzed using the sulfur filter since the metabolites, which contained only sulfur, could not be detected with the phosphorus filter. The samples were made up to the desired volume to obtain approximately the same peak area as that found in the linear portion of the curve. Peak area was plotted against nanograms of the insecticide injected. Table I (Poultry Feed and Egg Yolk) shows, respectively, sample size, retention time, and recoveries from three sample runs for GS-13005 for the 526-mμ phosphorus filter and 394-mμ sulfur filter.

Table II. Difference in Recoveries Obtained from Changes in Cleanup Procedures (Egg Yolk)

Sample	% Recoveries from No. 5 Column Only	% Recoveries from Florisil Column Only	% Recoveries from Use of Both Columns
1	88	80	80
2	90	79	86
3	92	84	91
4	94	80	89
Average	91%	81%	86%

Extraction of the egg yolk in the Omni-Mixer with petroleum ether was enhanced by grinding the sample with anhydrous sodium sulfate which increased the sample surface area by distributing the egg yolk onto the sodium sulfate and prevented gumming of the sample onto the sides and blades of the mixer.

The major part of the interfering materials was removed by acetonitrile partitioning and the No. 5 column (Samuel, 1966). The fatty material was removed by the acetonitrile partitioning while waxes and pigments were removed using the No. 5 column. After the acetonitrile partitioning was completed, the acetonitrile was washed with two additional 50-ml. portions of petroleum ether, removing the cloudiness found in the samples at that part of the cleanup procedures, resulting in higher recoveries of the pesticide. Recoveries of GS-13005 from egg yolk, shown in Table II, using either the Florisil column (81% recovery) or both the No. 5 and Florisil

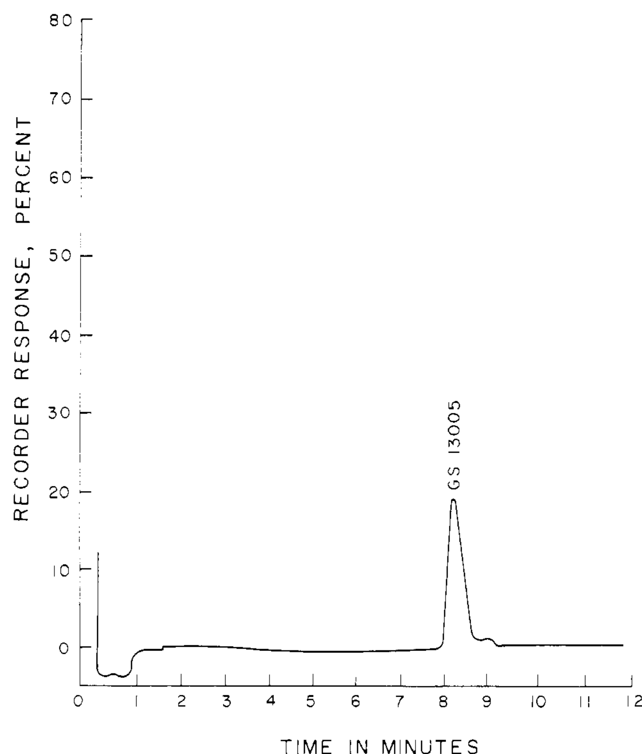

Figure 2. Gas chromatogram of GS-13005 (Supracide) with Florisil cleanup; no metabolites present

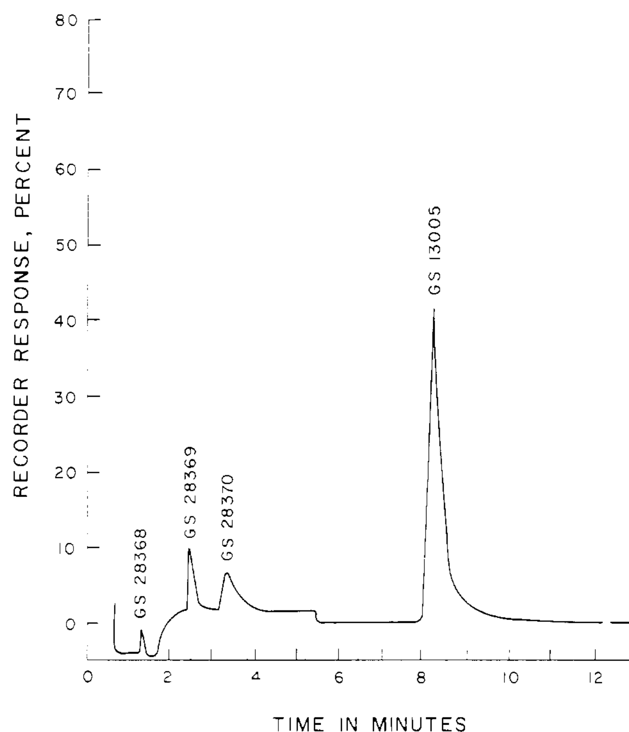

Figure 3. Gas chromatogram of GS-13005 (Supracide) without Florisil cleanup; metabolites present

columns (recovery 86%), were lower than when only the No. 5 column (91% recovery) was used for cleanup.

Figure 2 illustrates an actual chromatograph of GS-13005 extracted from egg yolk and cleanup on the Florisil column and the No. 5 column showing a clean chromatograph without interfering naturally occurring compounds or metabolites in the sample.

The sample used in Figure 3 was an extract of GS-13005 from egg yolk using only the No. 5 column showing GS-13005 and the metabolites illustrating the efficiency of the cleanup procedures used, without interfering natural products and the ability of the recorder to return to the baseline, a characteristic of clean samples.

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